



Missouri Department of Natural Resources

Village Creek Macroinvertebrate Community Assessment

In Response to the Critical Mineral Recovery Fire

Fredericktown, Missouri

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Prepared for:

**Missouri Department of Natural Resources
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1.0 Introduction

At the request of the Missouri Department of Natural Resources' (**MoDNR**) Environmental Remediation Program, staff from the Environmental Services Program's (**ESP**) Aquatic Bioassessment Unit conducted a biological assessment of Village Creek (**VC**) within the city limits of Fredericktown in Madison County, Missouri, after an October 30, 2024, fire at Critical Minerals Recovery (**CMR**).

CMR is a facility that recycles lithium-ion batteries used in electric vehicles. Runoff from fire suppression contained high concentrations of metals such as aluminum, lithium, and copper as well as the chemical components in fire-fighting foams. In addition, water used to fight the fire was chlorinated municipal water, which can be toxic to aquatic organisms. Fire suppression water flowed from the CMR facility into a road ditch that parallels State Highway OO. The ditch flows generally northward into an ephemeral tributary stream, which flows through a culvert under State Highway OO and continues roughly 0.25 miles northwest to Village Creek.

Acute effects on aquatic biota observed by MoDNR, Missouri Department of Conservation, and U.S. Fish and Wildlife Service personnel included dead fish, crayfish, frogs, and aquatic worms. These effects extended from the confluence of the tributary stream downstream to the mouth of VC. Dead aquatic animals were also observed in the Little St. Francis River for 0.6 miles downstream of the VC confluence to the Business 67 bridge. It is likely that particulate contaminants present in fire suppression runoff settled into the benthic sediment of VC, but whether persistent chronic effects exist in this environment is unknown. The purpose of this study was to assess the macroinvertebrate community after exposure to fire suppression runoff and products of combustion, and to observe if potential contamination of the substrate affected macroinvertebrates' ability to recolonize and inhabit the stream reach downstream of the tributary.

On December 12, 2024, Kenneth B. Lister and Evan A. Newman of MoDNR's Aquatic Bioassessment Unit collected macroinvertebrate samples from two sites for this project. VC 1.65 served as the upstream control station. It was located downstream of the County Road 211 bridge, approximately 0.3 miles upstream of the tributary that carried fire suppression runoff to Village Creek. VC 1.2 was approximately 0.1 miles downstream of the tributary. Coordinates for VC 1.65 were UTME 739421, UTMN 4162908, and coordinates for VC 1.2 were UTME 739025, UTMN 4162349. Both stations were in zone 15.

2.0 Methods

2.1 Macroinvertebrate Collection and Laboratory Processing

Macroinvertebrate samples were collected using a modified Hess Sampler (Caton and Chadwick 1984) consistent with MDNR-ESP-208, *Macroinvertebrate Sampling Using a Hess Stream Bottom Sampler* (MoDNR 2022a). A random number generator was used onsite to select seven sampling locations at each site. Each replicate sample was preserved, processed, and identified separately.

Steps to assure accuracy of organism removal from sample debris in the laboratory were performed consistent with those methods described in the *Semi-quantitative Macroinvertebrate Stream Bioassessment Project Procedure* (MoDNR 2024). In our experience with Hess samples, relatively little material is collected, so the decision was made *a priori* to process 100 percent of each replicate rather than subsample. Macroinvertebrates found in samples were identified to taxonomic levels specified in SOP MDNR-ESP-209, *Taxonomic Levels for Macroinvertebrate Identification* (MoDNR 2022b). Macroinvertebrate data were then entered into the Water Quality Monitoring Section's macroinvertebrate database consistent with SOP MDNR-ESP-214, *Quality Control for Data Processing* (MoDNR 2022c).

2.2 Macroinvertebrate Community Analysis

2.2.1 Primary Biological Metrics

Macroinvertebrate samples were analyzed using the following metrics: taxa richness, Ephemeroptera/Plecoptera/Trichoptera taxa, biotic index, and Shannon Diversity Index. These metrics were calculated for individual sample replicates for both VC stations following the *Semi-quantitative Macroinvertebrate Stream Bioassessment Project Procedure* (MoDNR 2024). The following is an explanation of the biological metrics used in this report.

- **Taxa Richness (TR)**

This measure reflects the health of the community through a count of the number of taxa present. In general, the total number of taxa increases with improving water quality, habitat diversity, and habitat suitability.

- **Ephemeroptera, Plecoptera, and Trichoptera Taxa (EPTT)**

This value summarizes taxa richness within the insect taxonomic orders that are generally considered to be pollution sensitive (Ephemeroptera [mayflies], Plecoptera [stoneflies], and Trichoptera [caddisflies]). The EPTT index generally increases with higher water quality.

- **Biotic Index (BI)**

This metric is a means of describing organic pollution tolerance (e.g. wastewater constituents) of individual taxa within the macroinvertebrate communities. It is expressed as a single value between 0 and 10, with 0 being the most sensitive and 10 being the most tolerant. The BI value for a macroinvertebrate sample is expressed as a weighted mean of all individuals identified in the sample.

- **Shannon Diversity Index (SDI)**

This index is a measure of community composition that considers both richness and evenness. It is assumed that a more diverse community is a healthier community. Shannon Diversity Index increases as the number of taxa increases and as the distribution of individuals among those taxa is more evenly distributed.

The use of Hess Samplers allows for the quantitative analysis of macroinvertebrate data. Statistical analysis of the primary biological metrics as well as the total number of

macroinvertebrate individuals (TI) was conducted using SigmaStat version 3.5 (Systat Software Inc. 2006). SigmaStat results sheets are presented in Appendix B.

Mean values of biological metrics were analyzed using the Student's t-Test with the threshold for statistical significance set at $p < 0.05$. For metrics whose normality test failed (TI), the nonparametric Mann-Whitney Rank Sum Test was used.

2.2.2 Secondary Biological Metrics

2.2.2.1 Quantitative Similarity Index

The Quantitative Similarity Index (QSI) was calculated to compare the macroinvertebrate community composition between stations. The QSI assesses two communities by comparing the presence and absence of macroinvertebrate taxa as well as their relative abundance (Shackleford 1988). This index can be used to assess a system's degree of impairment by establishing QSI threshold values or ranges when comparing test samples with controls. Shackleford (1988) developed scoring criteria in which a QSI > 65 percent indicates no impairment. Additional ranges below the 65 percent threshold include minimal impairment (QSI = 56-65 percent), substantial impairment (QSI = 45-55 percent), and excessive impairment (QSI < 45 percent).

2.2.2.2 Percent Sensitive Taxa

The BI metric, described in Section 2.1, was used to group macroinvertebrate taxa into five categories based on their sensitivity to pollutants. These categories ranged from extremely sensitive (BI values < 2.5) to extremely tolerant (BI ≥ 9.0). Sensitivity categories were calculated for each of the seven replicate samples collected per site and then averaged to provide a single percent sensitive taxa analysis for both VC sites.

2.2.2.3 Functional Feeding Groups

Macroinvertebrates also were grouped based on primary means of feeding. Functional feeding groups (FFGs) can be important indicators of food quality (MoDNR 2024) and respond differently to environmental stressors such as sedimentation (Rabeni 2005) and nutrient enrichment (Lemly 1982, Wallace 1996). The percent of each of five main FFGs was calculated for each replicate sample. The results were then averaged to observe differences between sites. The following five FFGs are typically most abundant in samples: filterer-collectors, gatherer-collectors, predators, scrapers, and shredders. A final "other" category was also included, but this group usually makes up a miniscule portion of the sample. FFG assignments were those made by Merritt et al. (2019).

2.2.3 Macroinvertebrate Community Composition

Replicate samples were pooled to create a single taxa list for each site. The respective VC community compositions were then compared with one another. This analysis included comparing the percent that mayfly, stonefly, and caddisfly taxa contributed to samples. The percent dominant taxa metric was also analyzed to determine what taxa groups contributed to each sample site. Finally, the composite taxa lists were compared to assess presence/absence and abundance patterns among individual taxa.

3.0 Results

3.1 Primary Biological Metric Results

Statistical results of macroinvertebrate abundance and biological metric are presented in Table 1. These results are also presented graphically in Figures 1-5.

There was a considerable difference in abundance between the two sampling locations. Whereas the total number of individuals ranged from 253-523 at VC 1.2, the upstream station generally had greater abundance, ranging from 146-1518. When combining the seven replicates, the total count for VC 1.2 was 2517 specimens, compared to 4688 at VC 1.65. Although the mean number of individuals at the upstream VC 1.65 station ($\bar{x} = 669.74$) was nearly two times higher than VC 1.2 ($\bar{x} = 359.57$) (Figure 1), the difference was not statistically significantly different ($p = 0.053$) (Table 1).

Means of the TR, EPTT, BI, and SDI biological metrics were also compared among stations (Table 1; Figures 2-5). Despite the relatively high macroinvertebrate abundance at VC 1.65, the mean TR ($\bar{x} = 46.57$) was nearly the same as VC 1.2 ($\bar{x} = 47.43$). The number of EPT taxa, however, was significantly higher ($p = 0.020$) at VC 1.65 ($\bar{x} = 12.86$) than VC 1.2 ($\bar{x} = 8.71$). The mean BI value of VC 1.65 was notably lower, and the mean SDI value was slightly higher than VC 1.2. Neither of these differences, however, was statistically significant (Table 1).

Table 1. Student's t-Test and Mann-Whitney Rank Sum Test Results for Village Creek Hess Samples

Station	Total Individuals ^a	TR ^b	EPTT ^b	BI ^b	SDI ^b
Village Creek 1.65	669.74 ±426.49	46.57 ±11.43	12.86 ±3.44	5.73 ±0.66	3.00 ±0.18
Village Creek 1.2	359.57 ±93.80	47.43 ±9.00	8.71 ±2.21	6.17 ±0.27	2.84 ±0.27
<i>p</i> -value	<i>p</i> = 0.053	<i>p</i> = 0.879	<i>p</i> = 0.020	<i>p</i> = 0.128	<i>p</i> = 0.226

Values presented are mean values ± standard deviation. Each station had $n = 7$ Hess Sampler replicates. Bold=significant difference ($p < 0.05$). a=non-parametric Mann-Whitney Rank Sum Test, b=parametric Student's t-Test.

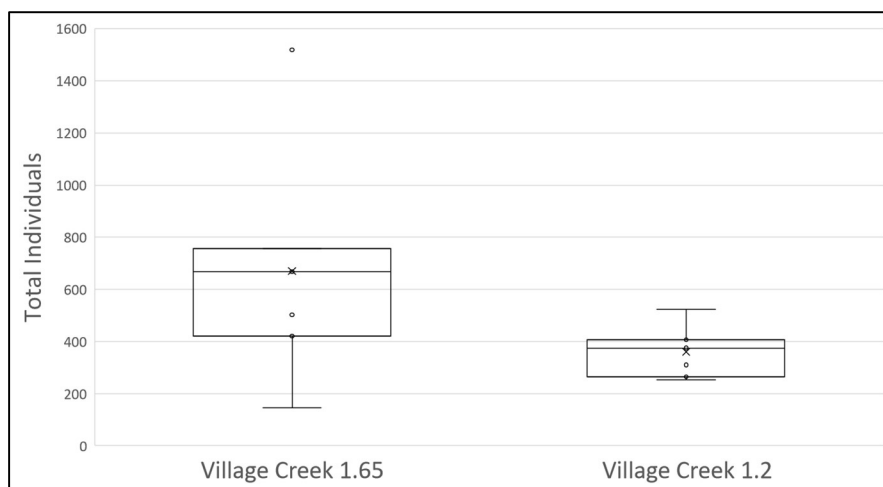


Figure 1. Box and whisker plot comparison of the total number of individuals collected in Village Creek Hess samples.

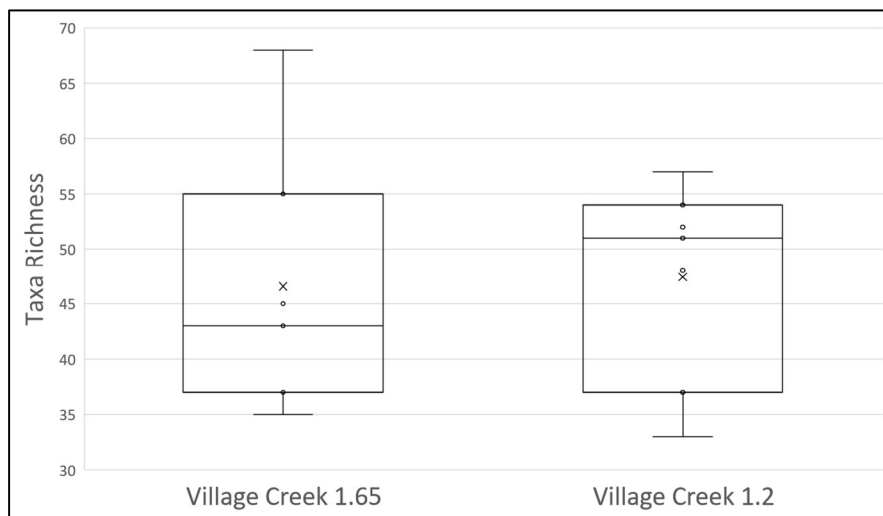


Figure 2. Box and whisker plot comparison of Village Creek taxa richness.



Figure 3. Box and whisker plot comparison of Village Creek EPT taxa richness.

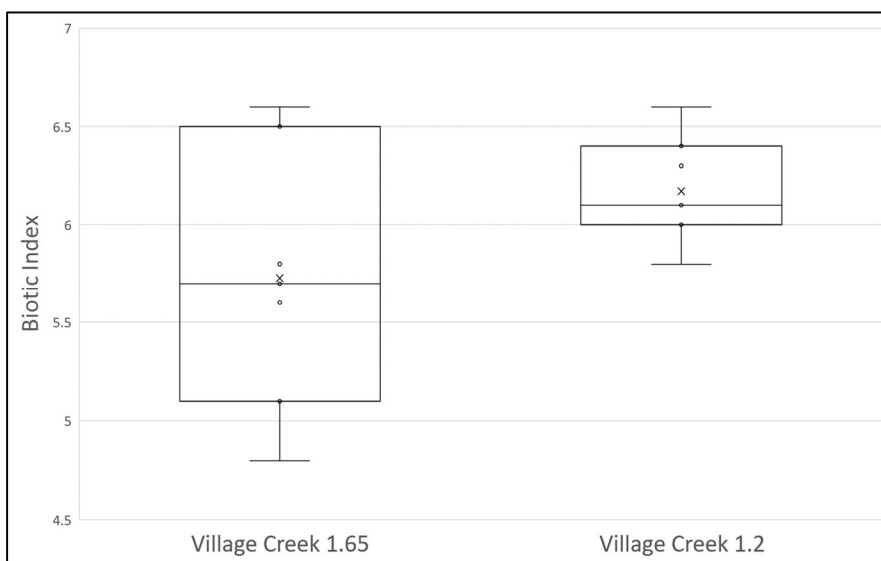


Figure 4. Box and whisker plot comparison of Village Creek biotic index values.



Figure 5. Box and whisker plot comparison of Village Creek Shannon Diversity Index values.

3.2 Secondary Biological Metrics

3.2.1 Quantitative Similarity Index

A QSI value was calculated for samples collected at VC 1.65 and VC 1.2 to determine the degree of macroinvertebrate community differences between stations. The QSI value calculated for the two VC sites was 44.9 percent. When gauged in terms of Shackleford's (1988) impairment thresholds, the downstream station is on the border between substantial impairment and excessive impairment.

A low QSI value is a result of two general factors: the number of taxa unique to one site or the other, and a large difference in the relative abundance of taxa. In the case of unique taxa, there were 36 taxa found only at VC 1.65 and 35 taxa found only at VC 1.2. Most of these unique taxa were relatively rare, each making up fewer than 5 individuals, but others were more abundant. Taxa that were numerous ($n > 10$) at VC 1.65 but absent at VC 1.2 included the riffle beetle *Microcyloepus glabratus*, two black fly genera (*Simulium* and *Prosimulium*), the stonefly *Isoperla*, and flat worms in the family Planariidae. Unique VC 1.2 taxa with >10 individuals included the chironomid *Cryptochironomus* and the mayfly *Neocloeon*. A weighted average BI calculation of unique taxa for each site showed that the taxa found only at VC 1.65 tended to be more sensitive (BI = 5.4) than the unique taxa of VC 1.2 (BI = 6.1).

Several taxa were present at both sites, but they contributed to the low QSI score by having large differences in proportional abundance. Caddisflies (mostly *Cheumatopsyche* and *Chimarra*) and isopods (*Caecidotea*) made up a sizeable portion of VC 1.65 samples, but they were rare at VC 1.2. Conversely, aquatic worms, water pennies, and some riffle beetles were abundant at VC 1.2 but relatively rare at VC 1.65. Among taxa that were present at both sites, the difference in relative abundance of these taxa was a large determinant of the low QSI value between VC sites. Taxa that exhibited proportional abundance differences between sites are discussed below in Section 4.3 (see also the bench sheet data in Appendix A).

3.2.2 Percent Sensitive Taxa

The BI value for each taxon collected at VC 1.65 and VC 1.2 was used to group macroinvertebrates based on their sensitivity to pollutants (Figure 6).

VC 1.65 had a much greater proportion of very sensitive ($BI < 2.5$) and a much lower proportion of very tolerant ($BI \geq 9.0$) taxa than VC 1.2. Even when combining categories to form a moderately sensitive group ($BI = 0 - 4.9$), VC 1.2 still had a much lower proportion in this group (21.9 versus 33.2 percent). When forming a moderately tolerant ($BI = 7.5 - 10$) group, however, the two stations are more similar (26.4 and 29.7 percent for VC 1.2 and VC 1.65, respectively).

VC 1.65 not only had a higher percentage of very sensitive taxa, but the number of taxa in this category was also considerably higher than VC 1.2. Whereas VC 1.65 had 12 taxa with BI values < 2.5 making up 4.6 percent of the sample, VC 1.2 had three taxa making up 0.3 percent. Most of the taxa making up the very sensitive group at VC 1.65 were EPT taxa, but the riffle beetle *Microcylloepus pusillus* was the most abundant among the 12 taxa with BI values < 2.5 .

Aquatic worms and a chironomid species group were the only representatives in the very tolerant group. Aquatic worms in the subfamily Tubificinae ($BI = 9.2$) were present at both sites, but they made up a much higher percentage of the VC 1.2 sample. Although *Polypedilum illinoense* grp. ($BI = 9.2$) was also found at VC 1.2, it was a minor component compared to Tubificinae. *Polypedilum illinoense* grp. was not collected at the upstream station.

As mentioned, when expanding the sensitive category to include taxa with BI values from 0 to 4.9, VC 1.65 still had more taxa representation and a higher percentage in this group. There were 24 taxa with BI values ≤ 4.9 in the VC 1.65 sample, but only 9 taxa at VC 1.2. Whereas two moderately sensitive taxa were abundant (> 100 individuals in the sample) at VC 1.2 (*Allocapnia* and *Psephenus herricki*), there were five moderately sensitive taxa at VC 1.65. Although most of the moderately sensitive taxa at VC 1.65 were EPT taxa (*Allocapnia*, *Chimarra*, and *Stenonema pulchellum*), two were chironomids (*Parametriocnemus* and *Eukiefferiella*).

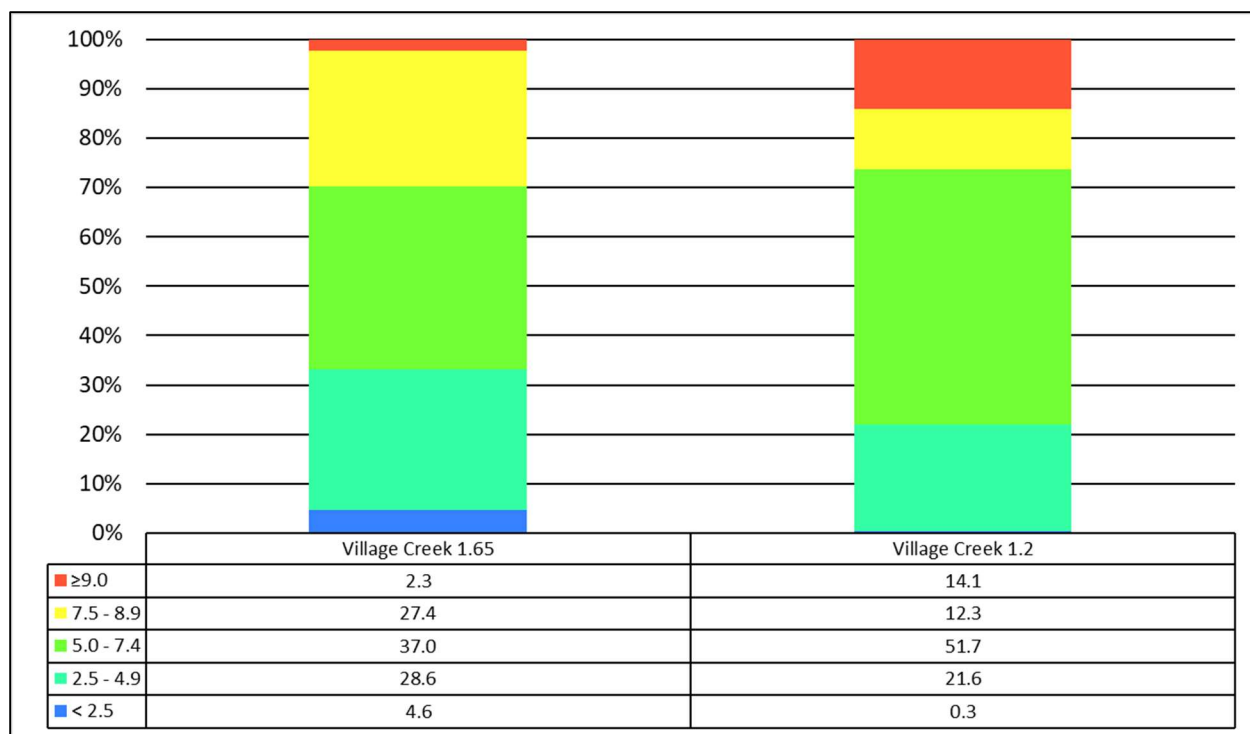


Figure 6. Percent sensitive taxa categories for Village Creek sampling locations.

3.2.3 Functional Feeding Groups

The five major FFGs made up similar percentages between VC sites, with the exception of filterer-collectors (hereafter referred to as filterers) and shredders (Figure 7). Although nine taxa were classified as filterers in VC 1.65 samples, two caddisfly taxa (*Cheumatopsyche* and *Chimarra*) and one chironomid (*Rheotanytarsus*) accounted for the majority. As discussed in Section 3.3 below, these caddisflies were very rare at VC 1.2. VC 1.2 had seven filterer taxa, but only two chironomid taxa (*Tanytarsus* and *Rheotanytarsus*) were represented by more than just a few ($n > 10$) individuals. Although the black flies *Simulium* and *Prosimulium* only made up about 1 percent of the VC 1.65 sample, these filterers were commonly present in replicates. They were not found, however, in the VC 1.2 sample. The mayfly *Isonychia bicolor* was another filterer that was abundant at VC 1.65 but rare at VC 1.2.

There was a nearly equal number of shredder taxa between stations, and the shredder taxa lists were similar. Also, the same two taxa (*Allocapnia* and *Cricotopus/Orthocladius*) were dominant in this FFG at both sites. However, the chironomid group *Cricotopus/Orthocladius* was far more abundant and made up a much larger percentage of the VC 1.2 sample compared to VC 1.65, and this chironomid group accounted for the overall difference in the shredder FFG percentage between sites.

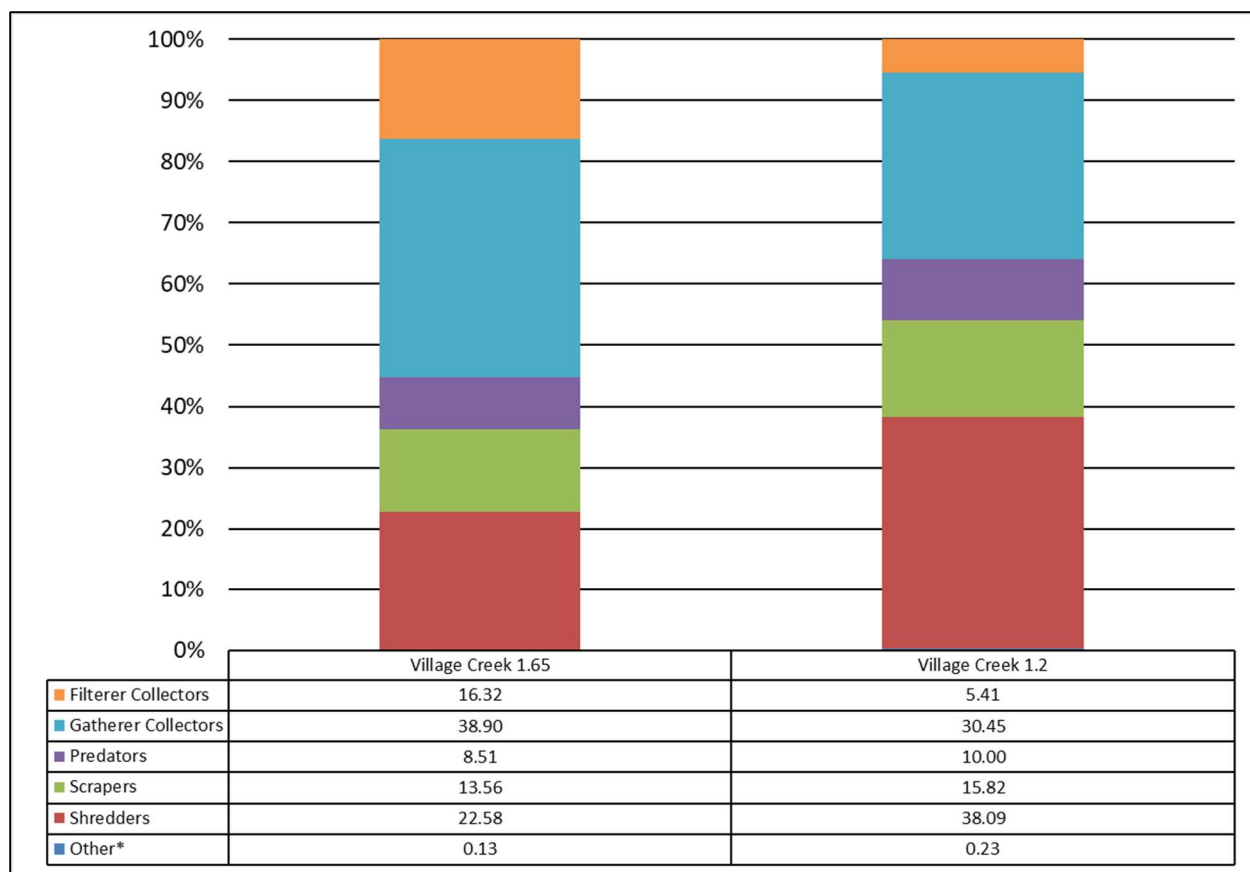


Figure 7. Percent functional feeding group categories for Village Creek sampling locations.
*“Other” includes parasites, macrophyte piercers, and taxa with no identified FFG.

3.3 Macroinvertebrate Community Composition

For community composition analyses, macroinvertebrate data from each site’s seven replicate samples were pooled to allow such metrics as TR, EPTT, and percent dominant taxa to be compared between stations.

Despite the large difference in abundance between sites mentioned in Section 3.1, the number of taxa present at each site was nearly identical (Table 2). The taxa making up this metric, however, differed between sites. Many taxa were present at only one station or the other, and several taxa that were present at both sites differed substantially in abundance.

There were several differences among EPT taxa between the two sites, chiefly that there were far fewer EPT taxa at VC 1.2 than VC 1.65. Mayflies were much more abundant at the upstream site ($n = 729$ versus $n = 155$), but both stations had 13 mayfly taxa. *Caenis latipennis* and *Stenonema pulchellum* were the dominant mayfly taxa at both sampling locations, but they were found in far greater abundance at VC 1.65 and accounted for a greater percentage of the sample.

Table 2. Village Creek Macroinvertebrate Community Composition

↓ Variable	Station →	VC 1.65	VC 1.2
TR		105	104
EPTT		32	21
% EPT			
% Ephemeroptera		15.5	6.1
% Plecoptera		9.4	11.9
% Trichoptera		10.3	0.4
% Dominant Families			
Chironomidae		39.5	50.3
Tubificida		1.0	13.4
Capniidae		9.0	11.5
Elmidae		4.9	7.8
Psephenidae		0.8	4.2
Heptageniidae		8.0	2.1
Caecidotea		11.1	0.6
Hydropsychidae		5.2	0.3
Philopotamidae		4.7	<0.1
% Dominant Taxa			
<i>Cricotopus/Orthocladius</i>		10.4	24.8
<i>Allocapnia</i>		9.0	11.5
Tubificinae		1.0	11.4
<i>Stenelmis</i>		1.4	7.7
<i>Psephenus herricki</i>		0.7	4.2
<i>Tanytarsus</i>		1.5	3.7
<i>Caenis latipennis</i>		5.0	2.9
<i>Thienemannimyia grp.</i>		4.9	2.6
<i>Caecidotea</i>		11.1	0.6
<i>Cheumatopsyche</i>		5.2	0.3
<i>Rheotanytarsus</i>		4.9	0.7
<i>Chimarra</i>		4.6	<0.1
<i>Stenonema pulchellum</i>		4.5	0.9

Stoneflies were slightly more numerous in the pooled VC 1.65 sample, but they made up a lower percentage of the sample compared to VC 1.2. Despite differences in absolute abundance, the number of stonefly taxa was similar between sites. *Allocapnia*, a winter stonefly, made up the majority of stonefly abundance and accounted for similar percentages at both stations. *Allocapnia* was the only stonefly taxon to be present in all seven replicates at both sites. All other stonefly taxa were represented by a few individuals ($n \leq 3$ at VC 1.2 and $n \leq 12$ at VC 1.65), and most were present in only one or two replicates.

Of particular note was the difference in both diversity and abundance of caddisflies between stations. Whereas VC 1.65 had 483 caddisfly individuals among nine taxa, VC 1.2 had only nine individuals among two taxa. The net-spinning genera *Cheumatopsyche* and *Chimarra* were the most abundant caddisflies at VC 1.65, both of which were represented by > 200 individuals. By comparison, there were eight *Cheumatopsyche* specimens and a single *Chimarra* individual at VC 1.2. In addition, VC 1.65 had seven caddisfly taxa that were found only at this site; however, each of these unique taxa was relatively rare, making up ≤ 7 individuals.

Similar to the net-spinning caddisflies mentioned above, there was a stark difference in the abundance of black fly larvae (Simuliidae) between stations. Black flies were not present in large numbers at VC 1.65, but they were found in most replicate samples. Although *Simulium* and *Prosimulium* combined to make up only 1.2 percent of the VC 1.65 sample ($n = 58$ individuals), they were absent from the VC 1.2 sample.

The number of chironomid taxa was slightly higher at VC 1.2, but they accounted for a much higher percentage of the sample than the upstream station (Table 2). Chironomid taxa tended to be more evenly represented at VC 1.65, with *Cricotopus/Orthocladius* being the only taxa group to account for >10 percent of chironomids. Five other chironomid taxa were relatively abundant, making 3 - 5 percent of chironomids. By comparison, *Cricotopus/Orthocladius* made up nearly 25 percent of VC 1.2 chironomids, and only one other taxon (*Tanytarsus*) made up >3 percent of chironomids.

Water pennies (Psephenidae) and riffle beetles (Elmidae) also were proportionally more abundant in the VC 1.2 sample. A single psephenid species, *Psephenus herricki*, made up 4.2 percent of the VC 1.2 sample; VC 1.65 had two psephenid taxa, but they only accounted for 0.8 percent of the sample. Similarly, five elmids made up 4.9 percent of the VC 1.65 sample, but the three elmids made up 7.8 of the VC 1.2 sample. Among riffle beetles, only *Stenelmis* was abundant at VC 1.2, but VC 1.65 had four elmids make notable contributions to the sample.

Non-insect taxa groups that exhibited large differences in abundance between sites included aquatic worms, flat worms, and isopods (Appendix A). Aquatic worms were much more abundant and diverse at VC 1.2. Station VC 1.2 had six aquatic worm taxa, making up over 13 percent of the overall sample. By comparison, VC 1.65 had four taxa making up about 1 percent of the sample. Interestingly, given that aquatic worms (Tubifida, Lumbricina, and Lumbriculida) were so abundant in VC 1.2, flatworms in the family Planariidae were absent. Although they did not account for a large proportion of the VC 1.65 sample (1.5 percent), they were found in every replicate. The isopod *Caecidotea* was rarely encountered in VC 1.2 replicate samples, but they were relatively abundant in VC 1.65 and ranked among the top five dominant taxa.

4.0 Discussion

4.1 Primary Biological Metrics

Despite the mean number of individuals at VC 1.65 being nearly twice as high as VC 1.2, there was no statistically significant difference between sites. It is likely that the high standard deviation at the upstream station resulted in a p -value that was slightly above the $p < 0.05$

threshold. The number of individuals among replicates at the VC 1.65 upstream control sample ranged from 146 to 1518, and the VC 1.2 downstream test sample ranged from 253 to 523. Aside from the two outliers that defined the range, the remaining replicate samples at VC 1.65 tended to have similar abundance. Mean TR values were nearly equal between stations but given that the mean number of EPT taxa was significantly higher at VC 1.65, it was apparent that there were macroinvertebrate community differences between the two sites despite very similar taxa counts. Neither BI nor SDI mean values were statistically different between sites, but the differences in these metrics nevertheless indicated a slightly more tolerant and less diverse community at VC 1.2 compared to the upstream station.

4.2 Secondary Biological Metrics

4.2.1 Quantitative Similarity Index

The QSI value calculated for the two VC sites was 44.9 percent. Based on Shackleford's (1988) impairment thresholds, a QSI of 44.9 percent indicates that the downstream station is on the border between substantial impairment and excessive impairment. These are the two most severe categories given in the study.

Food resources and the macroinvertebrates that use them tend to have patchy distributions in streams (Cummins et al. 2019), and even samples collected from the same habitats in the same stream reach can have seemingly low QSI values. Natural variation between samples collected within the same stream reach can yield QSI values of 65 percent (Shackleford 1988), but Rabeni et al. (1999) expected that duplicate samples will have QSI values of ≥ 70 percent. A QSI of 44.9 percent between VC 1.65 and VC 1.2 could be explained as partly due to macroinvertebrate patchiness if the low value was solely due to the large number of unique taxa at each site. However, the high abundance of tubificid worms and certain tolerant chironomid taxa at VC 1.2, coupled with reduced community features such as caddisfly and mayfly diversity and abundance suggests that a water quality issue is more likely than food resources or habitat to be the cause.

4.2.2 Percent Sensitive Taxa

Although VC 1.65 had a greater proportion of very sensitive taxa ($BI < 2.5$) and a low proportion of tolerant taxa ($BI \geq 9.0$) compared to VC 1.2, the latter station was by no means devoid of sensitive taxa. Some sensitive taxa were even more abundant at VC 1.2 than at VC 1.65, including water pennies (Psephenidae), riffle beetles (Elmidae), and certain stoneflies (Capniidae). Whatever water quality problems led to the fish kill of November 1, 2024, following the CMR fire, they did not result in a complete elimination of all sensitive macroinvertebrate taxa in VC downstream of the tributary. As discussed in the following sections, the macroinvertebrate community exhibited a varied and complex response to fire suppression runoff exposure.

The percent sensitive taxa metric is based on groupings of BI values, which are indicators of tolerance to the effects of pollutants in wastewater, including elevated nutrient concentrations and low dissolved oxygen (Hilsenhoff 1987). The VC macroinvertebrate community, however, was exposed to a different suite of pollutants such as metals, fire-fighting foam, chlorine, and unknown chemicals from burned materials. Therefore, the BI metric may not be the most

responsive community indicator for these types of pollutants. For example, studies have found that two mayfly species in the genus *Stenonema* both have sensitive BI values ($BI \leq 3$), but they respond very differently to metals in benthic sediment (e.g. MoDNR 2018).

4.2.3 Functional Feeding Groups

Scrapers, predators, and gatherer-collectors made up similar percentages of both VC sites. Percentages of shredders and filterer-collectors, however, were notably different between sites. The shredder FFG was dominated by two taxa at both stations—the chironomid group *Cricotopus/Orthocladius* and the stonefly *Allocapnia*. *Cricotopus/Orthocladius* made up a much larger percentage of the VC 1.2 sample and was the deciding factor in the difference between sites. Shredders tend to feed on allochthonous materials such as partially decomposed leaf litter and wood, and they serve an important role in breaking these materials down so that their nutrients can be made available to other aquatic trophic levels (Cummins et al. 2019). In terms of relative abundance, shredders accounted for nearly twice as much of the VC 1.2 sample compared to VC 1.65. Despite this difference in shredder relative abundance, this FFG made up a sizeable proportion at both sites. It appears that the contaminants resulting in the November fish kill did not hinder the ability of shredder chironomids to inhabit or recolonize the VC 1.2 reach. Likewise, the relative abundance of stoneflies in the shredder FFG was similar between stations, suggesting conditions in the VC 1.2 reach were sufficient to sustain this group.

Filterers were three times more abundant at VC 1.65 compared to the downstream site. Caddisflies of the genera *Cheumatopsyche* and *Chimarra* were primarily responsible for the difference in this FFG between sites, but black flies in the family Simuliidae also contributed to the difference.

Both caddisfly genera mentioned above are net spinners whose means of obtaining food is to construct silken nets in flowing water, which trap such suspended items as detritus, diatoms, and small invertebrates (Huryn and Wallace 2019, Wiggins 1996). Caddisflies then glean these items from the net. The stark paucity of this feeding guild at VC 1.2, especially when compared to its abundance at the upstream control, may be due to entrained debris in the fire suppression runoff being caught in these nets and consumed by caddisfly larvae. Filter feeding caddisflies are not selective in food resources and are as likely to ingest inorganic particles as organic debris (Huryn and Wallace 2019). If particles entrained in fire suppression runoff were caught in caddisfly nets and eaten, they could have had a profound negative effect on the caddisfly population downstream of the tributary. It is also possible that small invertebrates died as a result of fire suppression runoff, drifted downstream, and were caught in caddisfly nets and eaten. Hypothetically, consumption of these potentially contaminated food items could bioaccumulate and be a cause for the near absence of net spinning caddisflies at VC 1.2.

The black fly genera collected at VC 1.65 are also filter feeders and there is likely some overlap with net spinning caddisflies in the types of food items ingested. Rather than spinning nets, black fly larvae use specialized labral fans attached to their head capsules to filter particulate material from flowing water. Similar to the net spinning caddisflies, black fly larvae are nonselective of the food resources they filter (Huryn and Wallace 2019) and may have also ingested these

particles. The fact that not even a single black fly individual was found at VC 1.2 may be due to their filtering and ingesting contaminated food particles or debris originating from the CMR fire. The degree to which this debris may be toxic to black flies by ingestion is unknown, but it may help to explain their absence in VC 1.2 samples.

Despite the absence or near absence of the filter feeding taxa mentioned above, other filterers did make up roughly 5 percent of the VC 1.2 sample. However, this was considerably lower than the 16 percent at VC 1.65. Unlike VC 1.65, whose filter feeders were primarily caddisflies and black flies, VC 1.2 filter feeders were overwhelmingly chironomid taxa. The feeding strategy of chironomid filterers is somewhat different than trichopterans and simuliids. Filter feeding chironomids that were abundant at VC 1.2 (e.g. *Tanytarsus*) tend to live at the entrance of silken tubes, where they use salivary nets spread between their forelegs to capture entrained food particles (Walshe 1951). The diet of *Tanytarsus* larvae depends on instar, with earlier instars consuming small diatoms and later instars having a more diverse diet of detritus items (Ingvason 2004). Filter feeding chironomids present at VC 1.2 (*Tanytarsus*, *Rheotanytarsus*, and *Microtendipes*) are considerably smaller than the dominant filter feeding trichopterans and simuliids at VC 1.65, which likely affected the preferred particle size for each group. Whether it was preferred particle size, foraging behavior, or other factors that resulted in the relatively low abundance of filter feeders at VC 1.2 is unknown, but the difference in this guild between the two sites is notable.

4.3 Macroinvertebrate Community Composition

Given the extent of the fish kill observed after the fire in October and November 2024, it was surprising to find as much macroinvertebrate abundance and diversity in December, roughly six weeks later. Missouri Department of Conservation (MDC) biologists conducted a rapid assessment of VC macroinvertebrates on November 14, 2024 (Matt Combes, MDC, November 21, 2024, email). They identified macroinvertebrates to order and major groups, and biologists used descriptive terms to compare relative densities among sites (e.g. abundant > several > some > rare). MDC conducted this initial investigation 2 weeks after the fire and, although they observed definite community differences among their sampling locations, the VC reach downstream of the tributary did have macroinvertebrates inhabiting the area.

Both this study and MDC's rapid bioassessment showed a drastic decline in caddisflies from upstream to downstream of the tributary. MDC biologists described finding "several" trichopterans upstream of the unnamed tributary compared to "rare" downstream. A month later, our samples showed similar results. VC 1.65 had caddisflies making up over 10 percent of pooled samples, but they accounted for only 0.4 percent at VC 1.2. Not only were caddisflies present in low numbers at VC 1.2, but only two caddisfly taxa were found at this site. This was a drastic difference from just upstream, where nine caddisfly taxa were present. *Cheumatopsyche* and *Chimarra* were present in nearly equal abundance at VC 1.65 ($n > 200$ individuals). At VC 1.2, there were eight *Cheumatopsyche* individuals and a single *Chimarra* found in samples.

The high abundance of net spinning caddisflies at VC 1.65 coupled with their near absence at VC 1.2 suggests that, regardless of the exact mechanism, the fire and resulting runoff had a

strong negative effect on filterers. A possible explanation for the absence of net spinning caddisflies has been discussed (Section 4.2.3), but this does not account for the abundance of some sensitive taxa at VC 1.2 and the absence of others. Benthic macroinvertebrates are prone to drift, either to avoid predation, relocate to find food resources, or in response to high flow events or chemical spills (Huryh and Wallace 2019). Depending on behavior, body shape, habitat preference, and anatomical features, some macroinvertebrate taxa are more prone to drift than others. For example, in an experiment comparing stepwise versus abrupt increases in flow, Imbert and Perry (2000) found that *Cheumatopsyche* were less prone to drift in gradually increasing flows and were present in drift samples only in response to abrupt increases in flow.

The black fly taxa found commonly in VC 1.65 samples may be similarly resistant to drift. Unlike net spinning caddisflies, which use fixed retreats and woven silk nets, many genera of black flies use specialized comb-like labral fans on their head capsules to filter particles from flowing water. Rather than a fixed retreat, black flies use silken threads in conjunction with a ring of hooks at the base of the abdomen to affix themselves to hard substrates such as cobble and large woody debris. When they become dislodged, simuliid larvae can use the silk thread to return to their original location. *Simulium* and *Prosimulium* are often collected in fast-flowing water (pers. obs.) and typically drift in response to predators, ultraviolet radiation, changes in discharge, and becoming physically dislodged from the substrate (Adler and Currie 2019). Similar to net spinning caddisflies, it appears that black flies were eliminated from the VC 1.2 sample reach and recolonization had not yet occurred prior to our sampling.

Nearly 5 inches of rainfall occurred on November 5-6, 2024, in the Fredericktown vicinity (National Oceanic and Atmospheric Administration website), and the Little St. Francis discharge increased from just under 1.2 cubic feet per second (cfs) to almost 7000 cfs (USGS gage #07035000). This event would likely meet the criteria of “abrupt” described by Imbert and Perry (2000) and should have been sufficient to dislodge at least a portion of the aforementioned taxa that are less prone to drifting. It seems apparent that stream flows were sufficient to induce the type of macroinvertebrate drift that can alter the community structure (Smith et al. 2019), but net spinning caddisflies and black flies were conspicuously rare or absent at VC 1.2 while at the same time they were abundant (caddisflies) or common (black flies) just upstream. Whether this is due to conditions in this reach continuing to be unsuitable for these taxa to colonize or because of some other factor is unknown.

Moderately sensitive winter stoneflies of the genus *Allocaenia* (BI = 2.8) were abundant at both stations and even made up a slightly higher percentage of the VC 1.2 sample. No stoneflies were noted in MDC’s November 14, 2024, rapid bioassessment samples collected from Village Creek, but they were among the most commonly collected specimens in our samples. Most of the stonefly taxa collected during this study transition from their larval stage and emerge from streams as adults in January or February in Missouri (Poulton and Stewart 1991). Based on their body size and the condition of their wing pads, it appears that the specimens collected during this study were nearly mature larval specimens. *Allocaenia* have a “univoltine-fast” life history, which enables them to inhabit small, intermittent streams (Grubbs et al. 2006). Univoltine-fast taxa can avoid desiccation in two ways. First, they can migrate into the hyporheic zone

immediately after hatching where they may then undergo diapause during which time development is halted until stream flow resumes. Other taxa will engage in egg diapause, in which eggs do not hatch until suitable temperature and flow cues prompt them to do so (McRoberts and Grubbs 2021).

It is possible that stoneflies were not observed by MDC personnel in November, even among upstream control samples, because they had not yet migrated vertically from the hyporheic zone into surficial substrate and were too deep to be collected by kick net sampling. Given the rapid growth rate of univoltine-fast taxa like *Allocapnia*, it is also possible that they were too small to be observed without magnification in November. *Allocapnia* were also not present in samples collected from VC in March 2019 during an earlier bioassessment study (MoDNR 2023). For *Allocapnia* to be among the most abundant taxa collected during this December study implies that in VC they leave the hyporheos, complete the larval phase of their life cycle, and emerge as adults during a short one- or two-month period during the winter. Some species of stoneflies become more prone to drifting as they approach the end of their life cycles (Krueger and Cook 1981). Given the mature state of the specimens we collected, it was apparent they were entering the final phase of their larval stage and may have engaged in drifting behavior described by Krueger and Cook (1981). Either *Allocapnia* were deep in the hyporheic zone at VC 1.2 and were not subjected to acute toxicity of contaminants as the fish kill was occurring, or the specimens we collected were colonizers that had drifted into the reach from upstream.

5.0 Summary

1. Despite a fish kill that occurred in the VC study reach six weeks prior to this study, the downstream test station had a similar number of taxa compared to the upstream control station.
2. Statistical analyses were conducted on biological metrics calculated for quantitative Hess samples. Seven replicate samples were collected at each station to compare VC 1.2, the reach affected by fire suppression runoff, to VC 1.65, a station approximately 0.5 miles upstream.
 - a. Macroinvertebrates were much more abundant at VC 1.65, with the mean among replicates being nearly two times higher than VC 1.2. Due to high variability among replicates, however, the difference in the total number of individuals was not statistically different between sites ($p = 0.053$).
 - b. Mean differences of taxa richness, biotic index, and Shannon Diversity Index between sites were not statistically significant.
 - c. EPT taxa was the only biological metric with a statistically significant difference. The mean EPT taxa value for VC 1.65 ($\bar{x} = 12.86$) was significantly higher ($p = 0.020$) than VC 1.2 ($\bar{x} = 8.71$).
3. The Quantitative Similarity Index calculated between sites was 44.9 percent, which is on the border between Shackleton's (1988) substantial impairment and excessive impairment categories when comparing sites separated by a pollutant point source.
4. VC 1.65 had a much greater proportion of very sensitive ($BI < 2.5$) and a much lower proportion of very tolerant ($BI \geq 9.0$) taxa than VC 1.2. Most very sensitive taxa at VC

- 1.65 were EPT taxa, and very tolerant taxa present at both sites comprised tubificid worms and chironomids of the *Polypedilum illinoense* species group.
5. The relative proportions of shredder and filterer FFGs were notably different between sites. Shredders made up a higher percentage of VC 1.2 samples, and filterers were more abundant at VC 1.65.
 - a. An absence of black flies (Simuliidae) and a near absence of net-spinning caddisflies (Hydropsychidae and Philopotamidae) at VC 1.2 accounted for much of the difference in filterers between sites.
 - b. Shredder taxa were more equally represented between sites than filterers, but the chironomid group *Cricotopus/Orthocladius* was much more abundant at VC 1.2, which explained much of the difference in this FFG.
 6. A macroinvertebrate community composition comparison between sites yielded a number of differences.
 - a. Taxa richness was nearly equal, with VC 1.65 having only a single taxon more than VC 1.2, but the number of EPT taxa was considerably lower at the downstream station.
 - b. Chironomids, tubificid worms, water pennies, and some riffle beetles were much more abundant at VC 1.2, whereas heptageniid mayflies, net spinning caddisflies, and the isopod *Caecidotea* were more abundant at VC 1.65.
 - c. The net spinning caddisflies *Cheumatopsyche* and *Chimarra* were among the dominant taxa at VC 1.65, but they were nearly absent at VC 1.2.
 - d. Caddisfly differences between sites was not limited to net spinners. There was a large difference for all caddisfly taxa, with VC 1.65 having greater diversity and abundance compared to VC 1.2.

6.0 Conclusion

CMR fire suppression runoff included chlorinated water, firefighting foam, metals, and partially burned materials from the facility. In addition, firefighting runoff water exhibited high biochemical oxygen demand and high chemical oxygen demand as evidenced by samples collected by MDNR Environmental Emergency Response personnel on November 2, 2024. The primary unknown that led to this study was whether contaminants from the CMR fire would inhibit macroinvertebrates from recolonizing the affected reach of VC after the fish kill. The affected reach of VC had a taxa richness similar to the upstream control station and a diverse array of taxa, including some taxa that are sensitive to pollutants. Although this study has shown that macroinvertebrates are capable of inhabiting the affected reach, important components of the community were reduced or lacking. The number of EPT taxa was much lower at VC 1.2 than the upstream VC 1.65 control site, and the difference in filter feeding taxa between sites was substantial. There was also a notable difference in abundance between sites, with the average count among VC 1.65 replicate samples being nearly twice that of VC 1.2.

Macroinvertebrate community differences between VC sample sites like those listed above were almost certainly the result of CMR fire suppression runoff. The number of macroinvertebrate taxa and abundance of individuals at VC 1.2 was encouraging, however, given the extent of the fish kill that occurred weeks before this study. Whether the VC 1.2 community will adjust over

time to be more similar to the unaffected upstream reach is unclear. Most macroinvertebrates collected during this study complete their life cycles in a year. A rapid bioassessment in December 2025, similar to MDC's November 2024 survey, would aid in determining whether a year of macroinvertebrate reproductive cycles and drift is enough to reestablish an invertebrate community in the affected reach that is comparable to the upstream location.

7.0 References Cited

- Adler, P.H. and D.C. Currie. 2019. Simuliidae. Chapter 26 in Merritt, R.W., K.W. Cummins, and M.B. Berg. 2019. *An Introduction of the Aquatic Insects of North America, Fifth Edition*. Kendall Hunt Publishing Company, Dubuque, Iowa. 1480 pp.
- Cummins, K.W., R.W. Merritt, and M.B. Berg. 2019. Ecology and distribution of aquatic insects. Chapter 6 in Merritt, R.W., K.W. Cummins, and M.B. Berg. 2019. *An Introduction of the Aquatic Insects of North America, Fifth Edition*. Kendall Hunt Publishing Company, Dubuque, Iowa. 1480 pp.
- Grubbs, S.A., C.M. Thomas, B.T. Hutchins, J.M. Taylor. 2006. Life cycles of *Allocaonia recta* and *Leuctra* spp. (Plecoptera: Capniidae and Leuctridae) across a flow gradient in a central Kentucky karst headwater stream. *Southwestern Naturalist* 5(2): 321-332.
- Hilsenhoff, W.L. 1987. An improved biotic index of organic stream pollution. *The Great Lakes Entomologist* 20(1): 31-39.
- Huryon, A.D. and J.B. Wallace. 2019. Habitat, life history, secondary production, and behavioral adaptations of aquatic insects. Chapter 5 in Merritt, R.W., K.W. Cummins, and M.B. Berg. 2019. *An Introduction of the Aquatic Insects of North America, Fifth Edition*. Kendall Hunt Publishing Company, Dubuque, Iowa. 1480 pp.
- Imbert, J.B. and J.A. Perry. 2000. Drift and benthic invertebrate responses to stepwise and abrupt increases in non-scouring flow. *Hydrobiologia* 436: 191-208.
- Ingvason, H.R., J. Ólafsson, and A. Gardarsson. 2004. Food selection of *Tanytarsus gracilentus* larvae (Diptera: Chironomidae): an analysis of instars and cohorts. *Aquatic Ecology* 38: 231-237.
- Krueger, C.C. and E.F. Cook. 1981. Life cycles, drift, and standing stocks of some stoneflies (Insecta: Plecoptera) from streams in Minnesota, USA. *Hydrobiologia* 83: 85-92.
- Lemly, A.D. 1982. Modification of benthic insect communities in polluted streams: combined effects of sedimentation and nutrient enrichment. *Hydrobiologia* 87: 229-245.
- McRoberts, T.C. and S. Grubbs. 2021. Effects of stream permanence on stonefly (*Insecta, Plecoptera*) community structure at Mammoth Cave National Park, Kentucky, USA. *Biodiversity Data Journal* 9:e62242. <https://doi.org/10.3897/BDJ.9.e62242>.

Merritt, R.W., K.W. Cummins, M.B. Berg. 2019. *An Introduction of the Aquatic Insects of North America, Fifth Edition*. Kendall Hunt Publishing Company, Dubuque, Iowa. 1480 pp.

Missouri Department of Natural Resources (MoDNR). 2018. Biological Assessment and Metals Characterization Report. Center Creek (WBID 3203), Jasper County. Missouri Department of Natural Resources, Division of Environmental Quality, Environmental Services Program. P.O. Box 176, Jefferson City, Missouri. 65102. 40 pp. + Appendices.

MoDNR. 2022a. Standard Operating Procedure MDNR-ESP-208. Macroinvertebrate Sampling Using a Hess Stream Bottom Sampler. Missouri Department of Natural Resources, Environmental Services Program, P.O. Box 176, Jefferson City, Missouri 65102. 6 pp.

MoDNR. 2022b. Standard Operating Procedure MDNR-ESP-209. Taxonomic Levels for Macroinvertebrate Identifications. Missouri Department of Natural Resources, Environmental Services Program, P.O. Box 176, Jefferson City, Missouri 65102. 15 pp. + Appendices.

MoDNR. 2022c. Standard Operating Procedure MDNR-ESP-214. Quality Control Procedures for Data Processing. Missouri Department of Natural Resources, Environmental Services Program, P.O. Box 176, Jefferson City, Missouri 65102. 7 pp.

MoDNR. 2023. Biological Assessment and Metals Characterization Report. Fredericktown Area, Part 2: Little St. Francis River (WBID 2854), Village Creek (WBIDs 2863 and 2864), and Saline Creek (WBID 2859), Madison County. Missouri Department of Natural Resources, Division of Environmental Quality, Environmental Services Program. P.O. Box 176, Jefferson City, Missouri 65102. 84 pp. + Appendices.

MoDNR. 2024. Semi-quantitative Macroinvertebrate Stream Bioassessment Project Procedure. Missouri Department of Natural Resources, Environmental Services Program, P.O. Box 176, Jefferson City, Missouri 65102. 19 pp. + Appendices.

National Oceanic and Atmospheric Administration, National Weather Service. Online Weather Data website. <https://www.weather.gov/wrh/Climate?wfo=lsx>. Accessed on February 7, 2025.

Poulton, B.C. and K.W. Stewart. 1991. The stoneflies of the Ozark and Ouachita mountains (Plecoptera). *Memoirs of the American Entomological Society*, No. 38. 116 pp.

Rabeni, C.F., N. Wang, and R.J. Sarver. 1999. Evaluating adequacy of the representative stream reach used in invertebrate monitoring programs. *Journal of the North American Benthological Society* 18(2): 284-291.

Rabeni, C.F., K.E. Doisy, and L.D. Zweig. 2005. Stream invertebrate community function responses to deposited sediment. *Aquatic Sciences* 67: 395-402.

- Shackleford, B. 1988. Rapid bioassessment of lotic macroinvertebrate communities. Arkansas Department of Pollution Control and Ecology, Little Rock, Arkansas. 44 pp.
- Smith, A.J., B.P. Baldigo, B.T. Duffy, S.D. George, and B. Dresser. 2019. Resilience of benthic macroinvertebrates to extreme floods in a Catskill Mountain river, New York, USA: Implication for water quality monitoring and assessment. *Ecological Indicators* 104: 107-115.
- United States Geological Service Current Water Data for Missouri online database.
<https://waterdata.usgs.gov/mo/nwis/rt>. Accessed on February 7, 2025.
- Wallace, J.B. and J.R. Webster. 1996. The role of macroinvertebrates in stream ecosystem function. *Annual Review of Entomology* 41: 115-139.
- Walshe, B.M. 1951. The feeding habits of certain chironomid larvae (subfamily Tendipedinae). *Journal of Zoology* 121(1): 63-79.
- Wiggins, G.B. 1996. Larvae of the North American Caddisfly Genera (Trichoptera), Second Edition. University of Toronto Press Incorporated. Toronto, Ontario, Canada. 457 pp.

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Appendix A
Village Creek Hess Sampler Taxa List
December 12, 2024

Village Creek 1.65
Replicate a

ORDER: Observed Taxa	Count
"HYDRACARINA"	
Acarina	17
BASOMMATOPHORA	
Ancylidae	8
COLEOPTERA	
Microcylloepus pusillus	27
Psephenus herricki	4
Stenelmis	6
DIPTERA	
Ablabesmyia	1
Cricotopus/Orthocladius	95
Eukiefferiella	55
Hemerodromia	2
Parametriocnemus	16
Paratanytarsus	1
Polypedilum flavum	2
Prosimulium	5
Rheocricotopus	1
Rheotanytarsus	7
Simulium	12
Tanytarsus	2
Thienemanniella	4
Thienemannimyia grp	22
Tipula	1
Tvetenia bavarica grp	2
EPHEMEROPTERA	
Acentrella	1
Acerpenna	9
Caenis latipennis	5
Dipheter	1
Eurylophella	1
Isonychia bicolor	26
Stenacron	1
Stenonema pulchellum	74
Stenonema terminatum	21
ORDER: Observed Taxa	Count
ISOPODA	
Caecidotea	153

ODONATA	
Argia	2
PLECOPTERA	
Allocaenia	69
Taeniopteryx burksi	1
TRICHOPTERA	
Cheumatopsyche	36
Chimarra	56
TRICLADIDA	
Planariidae	10

Village Creek 1.65
Replicate b

ORDER: Observed Taxa	Count
"HYDRACARINA"	
Acarina	1
BASOMMATOPHORA	
Ancylidae	6
COLEOPTERA	
Macronychus glabratus	1
Microcylloepus pusillus	46
Psephenus herricki	5
Stenelmis	4
DIPTERA	
Brillia	2
Chironomidae	6
Cricotopus bicinctus	2
Cricotopus/Orthocladius	34
Eukiefferiella	49
Hemerodromia	5
Parametriocnemus	21
Paratanytarsus	6
Phaenopsectra	3
Polypedilum fallax grp	1
Polypedilum flavum	5
Prosimulium	1
Rheocricotopus	4
Rheotanytarsus	25
Simulium	27
Tanytarsus	2
Thienemanniella	5
Thienemannimyia grp	12
Tvetenia bavarica grp	2
EPHEMEROPTERA	
Acentrella	6
Acerpenna	4
Caenis latipennis	1
Isonychia bicolor	12
Stenacron	1
Stenonema pulchellum	38
Stenonema terminatum	8
Tricorythodes	1

ORDER: Observed Taxa	Count
ISOPODA	
Caecidotea	81
ODONATA	
Argia	2
PLECOPTERA	
Allocaonia	59
Taeniopteryx burksi	2
Taeniopteryx metequi	2
TRICHOPTERA	
Cheumatopsyche	51
Chimarra	105
Hydropsyche	1
TRICLADIDA	
Planariidae	27
TUBIFICIDA	
Tubificinae	1

Village Creek 1.65
Replicate c

ORDER: Observed Taxa	Count
"HYDRACARINA"	
Acarina	10
BASOMMATOPHORA	
Ancylidae	3
COLEOPTERA	
Microcylloepus pusillus	23
Optioservus sandersoni	2
Psephenus herricki	12
Stenelmis	24
DIPTERA	
Chironomidae	4
Cricotopus/Orthocladius	34
Eukiefferiella	13
Hemerodromia	6
Hydrobaenus	1
Parametriochnemus	10
Paratanytarsus	7
Polypedilum fallax grp	2
Polypedilum flavum	5
Rheocricotopus	1
Rheotanytarsus	9
Simulium	1
Tanytarsus	4
Thienemanniella	9
Thienemannimyia grp	43
Tipula	1
Trichoclinocera	2
Tvetenia bavarica grp	2
EPHEMEROPTERA	
Acerpenna	8
Caenis latipennis	13
Eurylophella	2
Isonychia bicolor	8
Stenacron	6
Stenonema femoratum	2
Stenonema pulchellum	26
Stenonema terminatum	5

ORDER: Observed Taxa	Count
ISOPODA	
Caecidotea	102
LUMBRICINA	
Lumbricina	2
ODONATA	
Argia	2
Gomphidae	1
PLECOPTERA	
Allocapnia	23
Neoperla	1
Perlidae	1
Taeniopteryx	1
TRICHOPTERA	
Cheumatopsyche	29
Chimarra	12
TRICLADIDA	
Planariidae	18
TUBIFICIDA	
Tubificinae	7
VENEROIDA	
Pisidiidae	5

Village Creek 1.65
Replicate d

ORDER: Observed Taxa	Count
"HYDRACARINA"	
Acarina	29
BASOMMATOPHORA	
Ancylidae	23
COLEOPTERA	
Dubiraphia	14
Microcylloepus pusillus	44
Optioservus sandersoni	1
Psephenus herricki	4
Stenelmis	16
DIPTERA	
Brillia	4
Ceratopogoninae	1
Corynoneura	5
Cricotopus bicinctus	13
Cricotopus/Orthocladius	152
Dicrotendipes	1
Diplocladius	1
Eukiefferiella	61
Eukiefferiella brehmi grp	1
Hemerodromia	5
Limnophyes	3
Micropsectra	1
Nanocladius	1
Parametriochnemus	100
Paratanytarsus	69
Pilaria	1
Polypedilum aviceps	6
Polypedilum fallax grp	2
Polypedilum flavum	12
Polypedilum illinoense grp	3
Polypedilum scalaenum grp	1
Potthastia	2
Prosimulium	4
Rheocricotopus	11
Rheotanytarsus	142
Simulium	7
Stempellinella	2

ORDER: Observed Taxa	Count
DIPTERA, continued	
Stenochironomus	2
Tanytarsus	29
Thienemanniella	15
Thienemannimyia grp	86
Tribelos	1
Trichoclinocera	4
Tvetenia bavarica grp	13
EPHEMEROPTERA	
Acerpenna	11
Caenis latipennis	105
Eurylophella bicolor	3
Isonychia bicolor	9
Stenacron	4
Stenonema femoratum	10
Stenonema pulchellum	39
Stenonema terminatum	1
Tricorythodes	2
ISOPODA	
Caecidotea	123
MEGALOPTERA	
Sialis	1
ODONATA	
Argia	4
PLECOPTERA	
Allocapnia	135
Isoperla	12
Strophopteryx	1
TRICHOPTERA	
Cheumatopsyche	95
Chimarra	38
Hydropsyche	6
Oecetis	1
Pycnopsyche	1
Rhyacophilidae	2
Triaenodes	1
TRICLADIDA	
Planariidae	3

Village Creek 1.65
Replicate d, continued

ORDER: Observed Taxa	Count
TUBIFICIDA	
Enchytraeidae	1
Tubificinae	13
VENEROIDA	
Corbicula	3
Pisidiidae	2

Village Creek 1.65
Replicate e

ORDER: Observed Taxa	Count
"HYDRACARINA"	
Acarina	11
BASOMMATOPHORA	
Ancylidae	2
COLEOPTERA	
Microcylloepus pusillus	1
Psephenus herricki	9
Stenelmis	6
DIPTERA	
Ablabesmyia	1
Ceratopogoninae	2
Chironomidae	1
Corynoneura	3
Cricotopus/Orthocladius	64
Dicrotendipes	3
Eukiefferiella	5
Hemerodromia	3
Hexatoma	1
Hydrobaenus	1
Myxosargus	1
Parametriocnemus	9
Paratanytarsus	21
Polypedilum flavum	2
Polypedilum scalaenum grp	1
Polypedilum tritum	1
Rheotanytarsus	4
Stempellinella	2
Tanytarsus	9
Thienemannimyia grp	32
Tvetenia bavarica grp	1
EPHEMEROPTERA	
Acerpenna	2
Caenis latipennis	59
Leptophlebiidae	3
Stenacron	11
Stenonema femoratum	25
Stenonema pulchellum	15

ORDER: Observed Taxa	Count
ISOPODA	
Caecidotea	58
LEPIDOPTERA	
Petrophila	1
MEGALOPTERA	
Sialis	1
ODONATA	
Argia	2
PLECOPTERA	
Allocaenia	18
TRICHOPTERA	
Cheumatopsyche	11
Chimarra	1
Helicopsyche	1
Rhyacophila	1
TRICLADIDA	
Planariidae	5
TUBIFICIDA	
Tubificinae	11

Village Creek 1.65
Replicate f

ORDER: Observed Taxa	Count
"HYDRACARINA"	
Acarina	16
AMPHIPODA	
Hyaella azteca	1
BASOMMATOPHORA	
Ancylidae	4
COLEOPTERA	
Dubiraphia	4
Ectopria nervosa	3
Psephenus herricki	1
Stenelmis	10
DIPTERA	
Brillia	3
Chironomidae	2
Corynoneura	10
Cricotopus/Orthocladius	73
Dicrotendipes	8
Eukiefferiella	3
Hemerodromia	4
Hydrobaenus	4
Limnophyes	2
Micropsectra	3
Microtendipes	1
Parametriocnemus	53
Paratanytarsus	43
Phaenopsectra	9
Polypedilum fallax grp	4
Polypedilum flavum	4
Polypedilum illinoense grp	5
Polypedilum scalaenum grp	1
Rheocricotopus	3
Rheotanytarsus	40
Simulium	1
Stempellinella	1
Stictochironomus	2
Tanytarsus	17
Telopelopia	1
Thienemannimyia grp	33

ORDER: Observed Taxa	Count
DIPTERA, continued	
Tipula	1
Tribelos	2
Trichoclinocera	2
Tvetenia	3
EPHEMEROPTERA	
Caenis latipennis	41
Isonychia bicolor	6
Leptophlebiidae	4
Stenacron	19
Stenonema femoratum	33
Stenonema pulchellum	17
ISOPODA	
Asellidae	6
MEGALOPTERA	
Sialis	3
ODONATA	
Argia	4
Enallagma	2
PLECOPTERA	
Allocaenia	104
Taeniopteryx burksi	1
TRICHOPTERA	
Cheumatopsyche	23
Chimarra	8
Pycnopsyche	3
TRICLADIDA	
Planariidae	2
TUBIFICIDA	
Branchiura sowerbyi	1
Tubificinae	14

Village Creek 1.65
Replicate g

ORDER: Observed Taxa	Count
"HYDRACARINA"	
Acarina	2
BASOMMATOPHORA	
Physella	1
DIPTERA	
Ablabesmyia	1
Chironomidae	2
Chironomus	5
Corynoneura	1
Cricotopus bicinctus	1
Cricotopus/Orthocladius	35
Dicrotendipes	12
Eukiefferiella	1
Hydrobaenus	2
Limnophyes	1
Microtendipes	1
Natarsia	1
Parametriocnemus	1
Paratanytarsus	6
Paratendipes	1
Phaenopsectra	1
Polypedilum illinoense grp	1
Pseudolimnophila	1
Rheotanytarsus	1
Stempellinella	1
Stictochironomus	9
Tanytarsus	6
Thienemannimyia grp	3
EPHEMEROPTERA	
Caenis latipennis	10
Isonychia bicolor	1
Stenacron	1
Stenonema femoratum	14
Stenonema pulchellum	3
Stenonema terminatum	1
ISOPODA	
Caecidotea	2

ORDER: Observed Taxa	Count
PLECOPTERA	
Allocaenia	13
TRICHOPTERA	
Pycnopsyche	1
TRICLADIDA	
Planariidae	3

Village Creek 1.2
Replicate a

ORDER: Observed Taxa	Count
"HYDRACARINA"	
Acarina	2
BASOMMATOPHORA	
Physella	1
COLEOPTERA	
Psephenus herricki	9
Stenelmis	3
DIPTERA	
Ablabesmyia	6
Ceratopogoninae	4
Chaetocladius	1
Chironomidae	1
Chironomus	3
Corynoneura	12
Cricotopus bicinctus	2
Cricotopus/Orthocladius	79
Cryptochironomus	4
Dicrotendipes	6
Eukiefferiella	4
Hemerodromia	1
Microtendipes	1
Nanocladius	1
Parakiefferiella	1
Paraphaenocladius	1
Paratanytarsus	16
Paratendipes	6
Phaenopsectra	13
Polypedilum flavum	2
Polypedilum illinoense grp	4
Pseudochironomus	1
Rheocricotopus	2
Rheotanytarsus	7
Stempellinella	2
Stictochironomus	5
Tanytarsus	26
Thienemanniella	2
Thienemannimyia grp	8
Tribelos	7

ORDER: Observed Taxa	Count
DIPTERA, continued	
Zavreliomyia	2
EPHEMEROPTERA	
Acerpenna	1
Caenis latipennis	16
Isonychia bicolor	1
Leptophlebia	2
Stenonema femoratum	2
Stenonema pulchellum	5
Stenonema terminatum	1
ISOPODA	
Caecidotea	4
MEGALOPTERA	
Nigronia serricornis	1
Sialis	1
ODONATA	
Argia	2
Enallagma	1
Hagenius brevistylus	1
PLECOPTERA	
Allocaonia	56
Taeniopteryx burksi	1
TUBIFICIDA	
Aulodrilus	3
Branchiura sowerbyi	15
Enchytraeidae	1
Tubificinae	12

Village Creek 1.2
Replicate b

ORDER: Observed Taxa	Count
"HYDRACARINA"	
Acarina	6
COLEOPTERA	
Psephenus herricki	10
Stenelmis	9
DIPTERA	
Ceratopogoninae	2
Chironomidae	3
Cricotopus bicinctus	3
Cricotopus/Orthocladius	71
Cryptochironomus	2
Dicrotendipes	3
Eukiefferiella	2
Hexatoma	1
Hydrobaenus	4
Natarsia	4
Parametriocnemus	7
Paratanytarsus	3
Polypedilum fallax grp	1
Polypedilum flavum	1
Rheotanytarsus	3
Stictochironomus	3
Tabanus	1
Tanytarsus	10
Thienemanniella	3
Thienemannimyia grp	13
Tribelos	1
Trichoclinocera	3
EPHEMEROPTERA	
Caenis latipennis	11
Stenacron	4
Stenonema femoratum	4
Stenonema pulchellum	5
Stenonema terminatum	2
ODONATA	
Argia	3
PLECOPTERA	
Allocapnia	38

ORDER: Observed Taxa	Count
PLECOPTERA, continued	
Perlidae	1
TRICHOPTERA	
Cheumatopsyche	1
Chimarra	1
TUBIFICIDA	
Branchiura sowerbyi	14
Tubificinae	11

Village Creek 1.2
Replicate c

ORDER: Observed Taxa	Count
"HYDRACARINA"	
Acarina	2
COLEOPTERA	
Dubiraphia	2
Helichus fastigiatus	1
Psephenus herricki	22
Stenelmis	20
DIPTERA	
Ablabesmyia	2
Ceratopogoninae	5
Chironomidae	4
Chironomus	1
Clinotanypus	1
Corynoneura	1
Cricotopus bicinctus	3
Cricotopus/Orthocladius	60
Cryptochironomus	5
Dicrotendipes	6
Diplocladius	1
Eukiefferiella	5
Hemerodromia	1
Hexatoma	4
Hydrobaenus	3
Microtendipes	1
Natarsia	2
Paratanytarsus	8
Paratendipes	1
Polypedilum flavum	2
Polypedilum scalaenum grp	2
Rheotanytarsus	2
Stenochironomus	1
Stictochironomus	9
Tanytarsus	17
Thienemanniella	2
Thienemannimyia grp	7
Trichoclinocera	1
EPHEMEROPTERA	
Acerpenna	2

ORDER: Observed Taxa	Count
EPHEMEROPTERA, continued	
Caenis latipennis	11
Ephemera simulans	1
Isonychia bicolor	1
Neocloeon	1
Stenacron	1
Stenonema femoratum	5
Stenonema pulchellum	2
ISOPODA	
Asellidae	5
LUMBRICINA	
Lumbricina	1
LUMBRICULIDA	
Lumbriculidae	3
ODONATA	
Argia	3
Dromogomphus	1
PLECOPTERA	
Allocapnia	25
TUBIFICIDA	
Branchiura sowerbyi	1
Enchytraeidae	1
Limnodrilus hoffmeisteri	3
Tubificinae	39

Village Creek 1.2
Replicate d

ORDER: Observed Taxa	Count
"HYDRACARINA"	
Acarina	4
BASOMMATOPHORA	
Physella	2
COLEOPTERA	
Psephenus herricki	34
Stenelmis	44
DIPTERA	
Ceratopogoninae	3
Cladotanytarsus	1
Cricotopus/Orthocladius	86
Cryptochironomus	6
Dicrotendipes	7
Eukiefferiella	6
Hexatoma	5
Hydrobaenus	5
Limnophyes	2
Limoniidae	1
Micropsectra	1
Microtendipes	2
Monodiamesa	1
Parametriocnemus	6
Paratanytarsus	4
Paratendipes	2
Pericomaini	1
Pilaria	1
Polypedilum flavum	1
Polypedilum illinoense grp	2
Polypedilum scalaenum grp	9
Procladius	1
Rheocricotopus	1
Rheotanytarsus	2
Stempellinella	2
Stenochironomus	3
Stictochironomus	9
Tanytarsus	18
Thienemanniella	2
Thienemannimyia grp	9

ORDER: Observed Taxa	Count
DIPTERA, continued	
Tribelos	1
Trichoclinocera	4
EPHEMEROPTERA	
Caenis latipennis	11
Neocloeon	5
Stenonema femoratum	1
ISOPODA	
Caecidotea	2
MEGALOPTERA	
Nigronia serricornis	1
Sialis	1
ODONATA	
Argia	1
Enallagma	1
PLECOPTERA	
Allocaenia	17
TRICHOPTERA	
Cheumatopsyche	1
TUBIFICIDA	
Branchiura sowerbyi	3
Tubificinae	75

Village Creek 1.2
Replicate e

ORDER: Observed Taxa	Count
"HYDRACARINA"	
Acarina	1
BASOMMATOPHORA	
Physella	1
COLEOPTERA	
Dytiscidae	2
Psephenus herricki	10
Stenelmis	26
DIPTERA	
Ablabesmyia	2
Ceratopogoninae	4
Chironomidae	4
Chironomus	2
Cladotanytarsus	2
Clinotanypus	1
Corynoneura	1
Cricotopus bicinctus	3
Cricotopus/Orthocladius	86
Cryptochironomus	3
Dicrotendipes	4
Eukiefferiella	6
Hexatoma	1
Hydrobaenus	1
Micropsectra	1
Microtendipes	1
Natarsia	1
Parametriocnemus	9
Paratanytarsus	5
Phaenopsectra	3
Pilaria	2
Polypedilum fallax grp	1
Polypedilum flavum	2
Polypedilum illinoense grp	4
Polypedilum scalaenum grp	9
Rheocricotopus	2
Rheotanytarsus	3
Stenochironomus	3
Stictochironomus	5

ORDER: Observed Taxa	Count
DIPTERA, continued	
Tanytarsus	14
Thienemanniella	2
Thienemannimyia grp	12
Tribelos	5
Tvetenia bavarica grp	1
EPHEMEROPTERA	
Caenis latipennis	4
Isonychia bicolor	2
Leptophlebia	1
Neocloeon	5
Procloeon	3
Stenonema pulchellum	8
Tricorythodes	1
ISOPODA	
Caecidotea	6
MEGALOPTERA	
Sialis	1
ODONATA	
Argia	1
Enallagma	1
PLECOPTERA	
Allocaenia	25
Taeniopteryx burksi	1
TRICHOPTERA	
Cheumatopsyche	2
TUBIFICIDA	
Branchiura sowerbyi	4
Enchytraeidae	1
Tubificinae	72
VENEROIDA	
Corbicula	2

Village Creek 1.2
Replicate f

ORDER: Observed Taxa	Count
BASOMMATOPHORA	
Ancylidae	1
COLEOPTERA	
Microcylloepus pusillus	1
Neoporus	1
Psephenus herricki	12
Stenelmis	41
DIPTERA	
Ablabesmyia	7
Ceratopogoninae	3
Cladotanytarsus	1
Cricotopus/Orthocladius	66
Cryptochironomus	1
Dicrotendipes	1
Eukiefferiella	4
Hemerodromia	1
Hexatoma	3
Microtendipes	1
Parametriocnemus	5
Pilaria	1
Stictochironomus	1
Sympotthastia	2
Tabanus	1
Tanytarsus	1
Thienemannimyia grp	6
Tribelos	1
Trichoclinocera	2
EPHEMEROPTERA	
Caenis latipennis	7
Stenonema femoratum	1
PLECOPTERA	
Allocaenia	30
Perlidae	1
Perlodidae	1
Taeniopteryx	1
TRICHOPTERA	
Cheumatopsyche	3

ORDER: Observed Taxa	Count
TUBIFICIDA	
Enchytraeidae	1
Tubificinae	44

Village Creek 1.2
Replicate g

ORDER: Observed Taxa	Count
"HYDRACARINA"	
Acarina	8
COLEOPTERA	
Dytiscidae	1
Psephenus herricki	9
Stenelmis	51
DIPTERA	
Ablabesmyia	1
Ceratopogoninae	4
Chironomus	3
Chrysops	2
Cladotanytarsus	2
Clinocera	1
Corynoneura	2
Cricotopus bicinctus	2
Cricotopus/Orthocladius	177
Cryptochironomus	1
Dicrotendipes	6
Eukiefferiella	8
Hemerodromia	1
Hexatoma	5
Hydrobaenus	2
Labrundinia	2
Natarsia	2
Orthocladius (Euorthocladius)	3
Parakiefferiella	1
Parametriocnemus	5
Paratanytarsus	7
Phaenopsectra	1
Polypedilum scalaenum grp	2
Polypedilum trigonum	1
Rheotanytarsus	1
Stempellinella	2
Stictochironomus	7
Sympotthastia	2
Tanytarsus	9
Thienemanniella	4

ORDER: Observed Taxa	Count
DIPTERA, continued	
Thienemannimyia grp	12
Tribelos	4
Zavreliomyia	1
EPHEMEROPTERA	
Acerpenna	1
Caenis latipennis	13
Eurylophella enoensis	2
Neocloeon	1
Stenacron	5
Stenonema femoratum	3
Stenonema pulchellum	3
ISOPODA	
Caecidotea	2
PLECOPTERA	
Allocapnia	99
Perlidae	1
Prostoia	1
Taeniopteryx	1
TRICHOPTERA	
Cheumatopsyche	1
TUBIFICIDA	
Branchiura sowerbyi	4
Tubificinae	34

Appendix B
Statistical Analysis Results Sheets
Biological Metric Comparisons between Control Station VC 1.65 and Test
Station VC 1.2

Taxa Richness: t-test

Thursday, January 23, 2025, 1:01:39 PM

Normality Test: Passed (P = 0.799)

Equal Variance Test: Passed (P = 0.528)

Group Name	N	Missing	Mean	Std Dev	SEM
VC 1.2 TR	8	1	47.429	8.997	3.401
VC 1.65 TR	8	1	46.571	11.429	4.320

Difference 0.857

t = 0.156 with 12 degrees of freedom. (P = 0.879)

95 percent confidence interval for difference of means: -11.121 to 12.836

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.879).

Power of performed test with alpha = 0.050: 0.050

The power of the performed test (0.050) is below the desired power of 0.800. Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

EPT Taxa: t-test

Thursday, January 23, 2025, 1:02:36 PM

Normality Test: Passed (P = 0.210)

Equal Variance Test: Passed (P = 0.789)

Group Name	N	Missing	Mean	Std Dev	SEM
VC 1.2 EPTT	8	1	8.714	2.215	0.837
VC 1.65 EPTT	8	1	12.857	3.436	1.299

Difference -4.143

t = -2.681 with 12 degrees of freedom. (P = 0.020)

95 percent confidence interval for difference of means: -7.510 to -0.776

The difference in the mean values of the two groups is greater than would be expected by chance; there is a statistically significant difference between the input groups (P = 0.020).

Power of performed test with alpha = 0.050: 0.629

Biotic Index: t-test

Thursday, January 23, 2025, 1:03:29 PM

Normality Test: Passed (P = 0.458)

Equal Variance Test: Passed (P = 0.086)

Group Name	N	Missing	Mean	Std Dev	SEM
VC 1.2 BI	8	1	6.171	0.275	0.104
VC 1.65 BI	8	1	5.729	0.663	0.250

Difference 0.443

t = 1.633 with 12 degrees of freedom. (P = 0.128)

95 percent confidence interval for difference of means: -0.148 to 1.034

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.128).

Power of performed test with alpha = 0.050: 0.210

The power of the performed test (0.210) is below the desired power of 0.800. Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

Shannon Diversity Index: t-test

Thursday, January 23, 2025, 1:04:09 PM

Normality Test: Passed (P = 0.517)

Equal Variance Test: Passed (P = 0.324)

Group Name	N	Missing	Mean	Std Dev	SEM
VC 1.2 SDI	8	1	2.843	0.274	0.104
VC 1.65 SDI	8	1	3.001	0.181	0.0685

Difference -0.159

t = -1.277 with 12 degrees of freedom. (P = 0.226)

95 percent confidence interval for difference of means: -0.429 to 0.112

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.226).

Power of performed test with alpha = 0.050: 0.107

The power of the performed test (0.107) is below the desired power of 0.800.

Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

Total Individuals: t-test

Thursday, January 23, 2025, 1:04:35 PM

Normality Test: Failed (P < 0.050)

Test execution ended by user request, Rank Sum Test begun

Total Individuals: Mann-Whitney Rank Sum Test

Thursday, January 23, 2025, 1:04:35 PM

Group	N	Missing	Median	25%	75%
VC 1.2 TI	8	1	375.000	275.500	401.500
VC 1.65 TI	8	1	668.000	441.250	736.250

Mann-Whitney U Statistic= 40.000

T = 37.000 n(small)= 7 n(big)= 7 P(est.)= 0.055 P(exact)= 0.053

The difference in the median values between the two groups is not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.053)